Associations Between Single Nucleotide Polymorphisms In *Lep* And *TFAM* With Growth And Fertility In UK Holstein – Friesian Dairy Cows

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Introduction

The recent decline in fertility and longevity of high producing dairy cows is an important problem that must be addressed to ensure the successful future of dairy enterprises. This has stimulated a wealth of research into improving breeding decisions to produce genetically superior animals that are more able to cope with the demands of modern farming practices. One line of research is the development of marker assisted selection, which involves the identification of genes and polymorphisms that affect economically important traits such as production, growth, fertility and longevity.

Growth is controlled by a complex series of interactions in the somatotrophic axis involving metabolism, the endocrine system and skeletal system. Poor growth is a significant cause of heifer culling prior to first calving (Brickell, Bourne, McGowan *et al*. 2009) and therefore, growth must be sufficient to ensure reproductive maturity is reached by 15 months of age, at the start of the service period (Wathes, Brickell, Bourne *et al*. 2008). However, heifers that grow very quickly have been shown to have poorer fertility during their first lactation (Ettema and Santos, 2004) and also have a shorter lifespan.

Leptin (of the *Lep* gene; BTA 4) is produced primarily by adipose tissue and plays a central role in energy homeostasis. Leptin is an important hormone in many systems including productivity, fertility and the skeletal system (Cornish, Callon, Bava *et al*. 2002).

Mitochondrial transcription factor A (*TFAM*; BTA 28) is an essential protein for the maintenance and biogenesis of mtDNA (Jiang, Kunej, Michal *et al*. 2005). Polymorphisms in this gene may therefore affect energy production and subsequently growth and fertility.

This study investigated single nucleotide polymorphisms (SNPs) in *Lep* and *TFAM* and their associations between size and fertility traits in the first and second lactation in UK dairy cows. The SNPs of *Lep* included UASMS1, UASMS2, A1457G, C963T (all located in the promoter (Nkrumah, Li, Yu *et al*. 2005; Liefers, Veerkamp, te Pas *et al*. 2005)); Exon2FB (located in exon 2 (Buchanan, Fitzsimmons, Van Kessel *et al*. 2002)) and A59V (located in exon 3 (Liefers, Te Pas, Veerkamp *et al*. 2003)). The SNPs of *TFAM* studied were TFAM1 and TFAM3 (location currently unavailable due to commercial interest).

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Materials and methods

Holstein-Friesian heifers (n = 444) born between August 2003 and October 2004 on 18 commercial UK dairy farms and 1 primarily research farm (with 3 groups) were recruited for this study. Each heifer was measured aged approximately 1 month (28 ± 0.8 days), 6 months (184 ± 0.8 days, pre-pubertal) and 15 months (452 ± 3 days, post pubertal at start of service period). Weight, height at withers (HT), crown rump length (CRL) and heart girth diameter were recorded at these time points. Fertility was measured during the first and second lactation. Age at calving (AC) was recorded. For animals that were served at least once, conception (CONCEIVED), number of services for conception (SERVICES), number of days to conception following calving (DC), the prevalence of the animal being in calf 100 days after calving (IC100) and the calving interval period (CI) were recorded. The number of records used in each analysis varied depending on the availability of information for the traits and SNPs. Samples of whole blood were taken from each animal and spotted onto Whatman FTA cards (Whatman International Ltd, Maidstone, UK) for DNA extraction. Genotyping of the SNPs was performed by Orchid Cellmark (Abingdon, Oxford, UK).

Statistical analyses. Preliminary analyses were carried out using a range of models. For the growth analyses, investigations into the type of curve (orthogonal vs. spline) and order of polynomial were conducted. Decisions on the final model were made based on the evaluation of the log likelihood and size of residual variance for each test. Ultimately, the model consisted of a 4^{th} order polynomial on age whereby animals were grouped according to their herd (1 to 21), year (2003 or 2004) and season (1 = March to May, 2 = June to August, 3 = September to November and 4 = December to February) of birth. The SNP was also fitted as a fixed effect with 3 levels. All known pedigree information for the preceding three generations for each heifer was included (n=2,251 animals) and a heifer permanent environmental effect was fitted to account for repeated measurements.

Fertility traits were analysed using a variety of mixed model equations, whereby fixed effects were generally herd-year-season of calving, covariate of age at calving, average milk production per day per heifer and the SNP. Fixed effects were only fitted if they made a significant contribution to the overall variance component ($P < 0.05$). A random animal effect was fitted in all instances to account for background additive genetic variance. Fertility traits were log transformed where necessary to improve their distribution. Both IC100 and CONCEIVED were class variables (1 = yes / 0 = no) and therefore were analysed using a binary model. All analyses were performed using ASREML v2.0 (Gilmour, Gogel, Cullis *et al*. 2006). A false discovery rate (FDR) was calculated to account for multiple testing, whereby significance was achieved at FDR q value < 0.10.

Results and discussion

The phenotypic measurements of the heifers are summarized in Table 1 and the genotype frequencies are reported in Table 2. Allele frequencies of all SNPs were distributed according to the Hardy-Weinberg equilibrium expected values. Analyses of the results revealed that UASMS1, Exon2FB and C963T were in close linkage disequilibrium ($r^2 = 0.95$

– 0.98) and therefore, only UASMS1 was carried forward for further analyses. Linkage between UASMS2, A1457G and A59V was however lower (r^2 values ranged between 0.04 – 0.33) and hence these SNPs were studied independently. Analyses of the TFAM SNPs also showed close linkage ($r^2 = 0.85$), and therefore only TFAM3 was studied further.

Results from the association analyses are presented in Table 3. Significant associations were found between UASMS1 and IC100 (lactation 1) and DC (lactation 2). Trends of association were reported with HT and CI in lactation 2. A1457G was significantly associated with HT, DC (lactation 2) and CI (lactation 2) whereas A59V was significantly associated with CRL and AC (lactation 1), and a trend of association was found with heart girth (*P < 0.10*). No associations were found with UASMS2. TFAM3 was significantly associated with all size measurements apart from weight, (where a trend was observed) and all fertility parameters in lactation 1 apart from AC. No associations were found in lactation 2, possibly because fewer records were analysed at this stage of the analysis due to death or culling of animals.

Table 1: Phenotypic measures[α]

Age	1 month	6 months (n = 444)	15 months
Weight (kg)	56 ± 0.7	175 ± 1.7	373 ± 2.4
HT (cm)	80 ± 0.2	104 ± 0.3	126 ± 0.3
CRL (cm)	94 ± 0.4	135 ± 0.5	169 ± 0.5
Girth (cm)	89 ± 0.4	131 ± 0.4	174 ± 0.5
Lactation	1 (n = 349)		2 (n = 244)
AC (days)	792 ± 5.3		1209 ± 8.4
CONCEIVED	0.93 ± 0.01		0.95 ± 0.01
SERVICES	2.25 ± 0.1		2.27 ± 0.1
DC (days)	129 ± 4.6		117 ± 4.4
IC100	0.44 ± 0.03		0.49 ± 0.03
CI (days)	409 ± 5.0		386 ± 4.5

[α] Values presented as mean ± standard error.

Table 2: SNP genotype frequencies

SNP	CC	CT	TT
UASMS1	0.17	0.48	0.35
UASMS2	0.74	0.24	0.02
C963T	0.37	0.47	0.16
Exon2FB	0.35	0.48	0.17
A59V	0.61	0.33	0.06
TFAM1	0.44	0.46	0.10
	GG	AG	AA
A1457G	0.30	0.47	0.23
TFAM3	0.49	0.44	0.07

Table 3: P-values for significant associations between SNPs and phenotypic traits

SNP	UASMS1		A1457G		A59V		TFAM 3	
Weight (kg)	-		-		-		0.073	
HT (cm)	0.062		0.017[a]		-		0.039[a]	
CRL (cm)	-		-		0.034		0.036[a]	
Girth (cm)	-		-		0.083		0.016[a]	
Lactation	1	2	1	2	1	2	1	2
AC (days)	-	-	-	-	0.025	-	-	-
CONCEIVED	-	-	-	-	-	-	0.050	-
SERVICES	-	-	-	-	-	-	0.009[a]	-
DC (days)	-	0.024[a]	-	0.004[a]	-	-	0.006[a]	-
IC100	0.023[a]	-	-	-	-	-	0.029	-
CI (days)	-	0.051	-	0.007[a]	-	-	0.011[a]	-

- = not significant (P > 0.10)

[a] = significant after FDR (q < 0.10)

Leptin regulates a wide array of biological processes such as feed intake, energy expenditure, body weight and reproductive functioning (Ingvartsen and Boisclair, 2001). Therefore, SNPs in *Lep* have the potential to influence many downstream activities. This study found significant associations between leptin SNPs with growth and fertility parameters. UASMS1 and A1457G are both located in the promoter region of the gene and therefore these SNPs may influence transcription, which would affect leptin synthesis. These SNPs also retain some significant associations after the calculation of FDR, thus improving the likelihood that true associations were observed. A59V is located in exon 3 and causes a replacement of the amino acid alanine with valine. This could alter the functionality of the leptin protein, and hence represents a mechanism by which this SNP may affect growth and fertility.

These results also highlight the previously unidentified associations between *TFAM* SNPs with growth and fertility traits in dairy cattle, many of which were still significant after the calculation of FDR. TFAM has an essential role in regulating mitochondrial function and energy production and therefore it can be hypothesised that SNPs in this gene may have significant implications on many biological processes.

Conclusion

In conclusion, this study has highlighted significant associations between *Lep* and *TFAM* SNPs with growth and fertility traits, suggesting that these SNPs may be useful in marker assisted selection. Despite this, there have been no other studies involving *TFAM* SNPs in relation to growth or fertility and therefore further studies are required to verify these results.

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